

## THE EFFECT OF BREAST-FEEDING ON CRYPTOSPORIDIUM INFECTION RATE IN CHILDREN BELOW TWO YEARS AGE

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### ABSTRACT

*Cryptosporidium* spp. is a coccidian protozoan parasite that is now well recognized as a worldwide cause of diarrhea. Current study involved of 200 stool samples collected from children, who were hospitalized because of diarrhea. The direct smear method and then formalin-ether sedimentation were carried out to detect the intestinal parasites. Fecal smears were prepared from the sediment and stained by the modified Ziehl-Neelsen method for the recovery of acid-fast oocysts of *Cryptosporidium*. The study results showed that 18% of the hospitalized diarrheal children were infected with *Cryptosporidium* infection. The rate of infection in children who have breast-fed and mixed were low compared to children who have bottle-feeding the highest rate of infection in children were in the first three months of age, and the male more than female in the infection rate. It is concluded that the breast-feeding was demonstrated to reduce *Cryptosporidium* diarrhea in children and should be encouraged.

**KEYWORDS:** Breast Feeding Importance, Infection Rate for Children Below Two Year

### INTRODUCTION

*Cryptosporidium* is a protozoan parasite that infects the gastrointestinal tract of a wide range of vertebrates including humans, livestock, wild animals and birds<sup>1</sup>. The infective stage oocysts are highly resistant to chlorination, common household disinfectants and survive long periods in the environment (2). *Cryptosporidium* spp. Is a main pathogen causing acute diarrhea, nonspecific signs including dehydration, fever, anorexia, and weakness may be accompanied (3). It is characterized by self-limiting gastroenteritis in otherwise healthy people, while it is more severe in immunocompromised subjects and constitutes a serious threat leading to chronic or fulminant disease, wasting, and death<sup>4,5</sup>. In developing countries, *Cryptosporidium* is responsible for 8–19% of cases of diarrheal disease (1, 6), with a significant mortality rate (9). Children less than 12 months of age were most likely to cryptosporidiosis, and infection was significantly associated with diarrhea, vomiting, that need for hospitalization (8). A survey on the prevalence of cryptosporidiosis among human immunodeficiency virus (HIV) – positive were 73.6% at Mulago Hospital in Uganda while, HIV-negative children were 5.9% rate of infection (9).

The aim of this study is to determine the effect of breast-feeding on *Cryptosporidium* infection rate in children below two years age.

### MATERIALS AND METHODS

Two hundred stool samples were collected from hospitalized children (110 males & 90 females) suffering from unknown cause of diarrhea. All patients were hospitalized in the Basrah (south of Iraq) General Hospital, Zubair General Hospital for children in the province of Basrah. The study enrolled from November 2013 to March 2014.

## CONCENTRATION TECHNIQUE

After direct smear method examination, Ritchie formalin-ether sedimentation technique was used as a concentration technique according to Fleck and Moody (1988) For all participants in the study.

## PROCEDURE

- Fecal specimens about the size of pea on the end of swab stick was taken and emulsified with 7ml of 10% formal-saline.
- The emulsion was filtered through two layers of surgical gauze into a 15ml centrifuge tube.
- Three ml of ether was added to the filtrate, the mixture was thoroughly shaken for 1min.
- Centrifugation at 2000 round/min was made for shacked mixture.
- Four layers resulted; by ringing the "plug" (between formalin and ether layer) with an applicator stick to be loosen.
- Then proper decanted of the supernatant was made the deposit was mixed with the few drops remained in the tube.
- One or two drops of deposit was transferred on to a glass microscope slide and covered with cover slip which was examined by X10 objective, turned to the X40 or X100 lens for confirmation of identification of parasites. (That step record as wet mount smear after concentration method) except for human samples.
- The remained deposit was poured on to another glass slide to be subject of staining.

## STAINING TECHNIQUE

Modified acid-fast stain method was used for all stool samples according Beaver and Jung (1985).

## REAGENTS

- Kinyoun's Carbol-fuchsin: Basic fuchsin 4gram and phenol 8 gram.  
95% ethanol 20ml
- D. water 100 ml 2- Acid alcohol: 3ml of concentrated HCL was added to 97ml of 95% ethanol.
- Methylene blue: 1gram of methylene blue was dissolved in 100ml of 95% ethanol.

## PROCEDURE

- The slide of step 8 in concentration technique was allowed to dry and fixed in 95% methanol for 3 minutes.
- Covered with Kinyoun' s Carbol-fuchsin for 3 minutes.
- Rinsed with tap water.
- The slide was covered acid-alcohol for 1 minute.
- Washed with tap water.

- Methylene blue was used for 2 minutes, then washed and drained with gentle heat.
- Examination by microscope using low power turned to high oil power.
- Seeking for red-pink oocysts.

## RESULTS

*Cryptosporidium* infection rate for age in children indicate the highest percentage occur in (> 2month-3 month) old 35.71% while the lowest percentage occur in (> 5 month – 7 month) old 5% (Table 1). While the rate of *Cryptosporidium* infection in children according to the sex Indicate the male more than female in the infection rate, 23.63% in male and 11.11% in female (Table 2). Also found the rate of *Cryptosporidium* infection in children according to the type of feeding indicate The lowest percentage occur in breast-feeding children while the highest infection occur in bottle-feeding infants, 16.66% in breast-feeding, 22.22% in mixed-feeding and 61.11% in bottle-feeding respectively (Table 3).

**Table 1: *Cryptosporidium Parvum* Infection Rate for Age in Children**

Age	Number of Examined Samples	Number of Positive Samples	% of Infection
7 day – 2 month	18	4	22.22
> 2 month – 3 month	42	15	35.71
> 3 month – 5 month	52	6	11.53
> 5 month – 7 month	40	2	5
> 7 month – 10 month	36	6	16.66
> 10 month – 1 year	4	1	25
> 1 year – 2 year	8	2	25
<b>Total</b>	<b>200</b>	<b>36</b>	<b>18</b>

**Table 2: The Rate of *Cryptosporidium Parvum* Infection in Children According to the Sex**

Sex	Total Number	Number of Positive Samples	% of Infection
<b>Males</b>	110	26	23.63
<b>Females</b>	90	10	11.11
<b>Total</b>	<b>200</b>	<b>36</b>	<b>18</b>

**Table 3: The Rate of *Cryptosporidium Parvum* Infection in Children According to the Type of Feeding**

Type of Feeding	Total Number	Number of Positive Samples	% of Infection
<b>Breast - feeding</b>	64	6	16.66
<b>Bottle – feeding</b>	92	22	61.11
<b>Mixed – feeding</b>	44	8	22.22
<b>Total</b>	<b>200</b>	<b>36</b>	<b>18</b>

## DISCUSSIONS

The results of this study for the proportion of total infection in children (18%) comparable to other study in Basrah<sup>12</sup> and in Zambia<sup>13</sup> which was 18.3%, 21%, respectively is relatively agree, but they did not agree with the results in Iran (14) and in Peru (15), which were amounted to 7.66%, 6.4%, respectively.

Through the study found that the highest rate of infection in children were in the first three months of age.

The reason of diarrhea may be due to insufficient immunity in children because of; malnutrition in some infants; leave breast-feeding; and the use of bottle-feeding. The age of children play important role in diarrheal disease, the more younger is more liable to infection than the older children and this may be related to immunological status in old children. This mean there is inverse relation between the age and the infection, a matter which agree with other many studies (16, 17, 18).

While the rate of *Cryptosporidium* infection in children according to the sex Indicate the male more than female in the infection rate. The reason is probably neonate and early infancy the males are more liable to infection than females<sup>19</sup>.

Breast-feeding provides significant protection against many diseases including diarrhea in infancy<sup>20</sup>. Breast-fed infants have a lower prevalence of gastrointestinal infections including intestinal protozoan infections (IPI) than formula- fed infants (21, 22). In this study appeared the proportion of breast-feeding and mixed low compared to bottle-feeding, the reason that breast-feeding is available where properties not found in bottle-feeding of these characteristics that mother's milk is sterile, ready, suitable temperature as well as the mother's milk contain antibodies (IgA, IgG) which protects the infant from infection in addition to the ignorance of many of the mothers to matters sterilization and cleanliness of the water in bottle-feeding, the results of this<sup>(23,24)</sup>.

## CONCLUSIONS

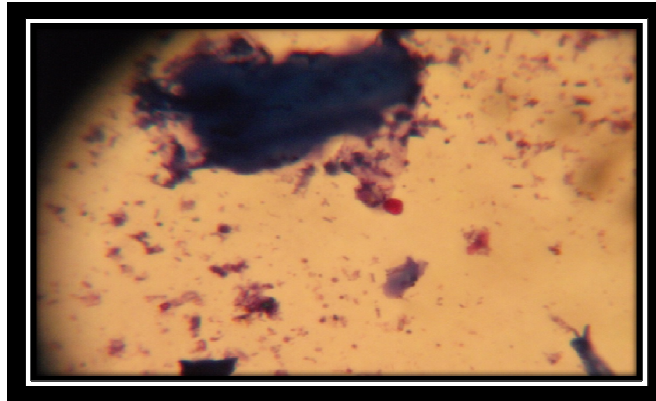
The results of this study offer confirmation for the protective effective of breast - feeding regarding cryptosporidiosis in children below two years . Breast-feeding should be encouraged, especially in areas where cryptosporidiosis is prevalent

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**Figure 1: Oocyst of Cryptosporidium Parvum Stained by the Modified Acid-Fast Method (100X)**